

Er:YAG Laser Activation of Sodium Hypochlorite for Root Canal Soft Tissue Dissolution

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Background and Objective: The aim of this *in vitro* study was to investigate the effect of Er:YAG laser irradiation on the ability of sodium hypochlorite (NaOCl) to dissolve soft tissue during endodontic procedures.

Materials and Methods: Two acrylic glass plates, each containing a semi-canal, were bolted together to form a complete canal. This geometry permitted one semi-canal to be filled with fine liver sausage of bovine origin dyed by methylene blue and the other with NaOCl (4.00–4.99% available chlorine; Sigma–Aldrich Corporation, St. Louis, MA), which was then activated by Er:YAG laser irradiation (KEY Laser 3; KaVo, Biberach, Germany) using a plain-ended fiber tip and a range of output energy and repetition rate. To achieve relatively low output energy from high input energy, the laser beam was attenuated by placing glass slides in the beam path. The resultant images acquired were analyzed using pixel-based analysis. Samples were statistically analyzed (two-way ANOVA, $P < 0.05$, univariate, bifactorial; IBM SPSS Statistics 19, SPSS Inc., Chicago, IL).

Results: Both output energy and repetition rate significantly influenced the tissue dissolution ability of NaOCl ($P < 0.05$).

Conclusion: Within the limitations of this *in vitro* study, we conclude that laser activation of NaOCl at 200 mW output power leads to effective soft tissue dissolution. This finding can be of use to endodontists pursuing effective soft tissue dissolution from their irrigants. *Lasers Surg. Med.* 45:339–344, 2013. © 2013 Wiley Periodicals, Inc.

Key words: cavitation; irrigant; Er:YAG laser

INTRODUCTION

In endodontic therapy, removal of soft tissue in the root canal is critical in eliminating nutritive substances for residual microorganisms. Because of its superior tissue-dissolving and bactericidal abilities, sodium hypochlorite (NaOCl) is the widely-preferred endodontic irrigant [1–4]. However, failure of endodontic therapy is commonplace in clinical practice because of the difficulty in achieving appropriate chemo-mechanical cleaning throughout the entirety of the complex root canal anatomy (curvatures, lateral branches, and apical ramifications) [5]. This results in residual nutritive substances and residual microorganisms

in the root canal. There is thus a continuing demand for adjuvant or alternative methods.

Efforts have been made to achieve sonic and ultrasonic activation of irrigants. Compared with traditional syringe irrigation, an improvement in root canal cleaning could be detected for ultrasonically activated NaOCl because of agitation or temperature rise [4,6,7], but the results are inconsistent [4]. Furthermore, there is a lack of certainty about the nature of the effects: the role of cavitation remains controversial [4,7].

Another field of interest is the application of laser technology to endodontics. A multitude of studies have evaluated the bactericidal effect of radiation with different laser systems. Except for *Enterococcus faecalis*, lasers can reduce bacteria below detectable levels [8–15]. When combined with endodontic irrigants, even *E. faecalis* was susceptible to lasers [15–17].

Laser systems with wavelengths strongly absorbed in water are of great interest. The Er:YAG-Laser produces a beam of light with a wavelength (2.94 μm) whose extinction coefficient in water is maximal [18]. Further investigations have demonstrated that different laser systems can create fluid motion [19–22] and that laser-activated irrigation can remove dentin debris [6,23,24]. However, these investigations have not extended to explore soft tissue dissolution by laser-activated irrigation. Thus, the aim of the study was to investigate the effect of Er:YAG laser irradiation on the *in vitro* soft tissue-dissolving ability of NaOCl. To our knowledge, this is the first description of this type of experimental set-up in the literature.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

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MATERIALS AND METHODS

Root Canal Model

The developed root canal model consisted of two acrylic glass plates, each with a semi-canal that, when combined, formed a complete canal (diameter: 1.5 mm, length: 20 mm). This geometry enabled us to fill one semi-canal with a soft tissue formulation (fine liver sausage of bovine origin, used because of its homogeneous and brushable consistency) and the other with NaOCl (4.00–4.99% available chlorine; Sigma–Aldrich Corporation, St. Louis, MI), to be activated by laser irradiation. Tissue of bovine origin has been extensively used previously for tissue dissolution [3,25,26]. The liver sausage was dyed with methylene blue (Merck, Darmstadt, Germany) to enhance the visibility of tissue dissolution.

Experimental Setup

Preliminary studies were performed to optimize the testing procedures. After filling one semi-canal with dyed soft tissue substitute using a fine spatula, the acrylic plates were screwed together. The empty semi-canal was then carefully filled with NaOCl using a syringe (diameter: 0.5 mm) without disturbing the tissue.

The root canal model was fixed upright on a positioning device. An endodontic handpiece with a fiber tip was fixed to a second positioning device. This setup (Fig. 1) allowed only vertical movements of the fiber tip inside the canal during the experimental procedures. The study was performed at room temperature.

Laser Treatment

An Er:YAG laser (wavelength: 2.94 μm , no air or water; KEY Laser 3; KaVo, Biberach, Germany) was used with an

endodontic handpiece (KaVo 2062; KaVo) and a plain-ended fiber tip made of silica (diameter: 300 μm). As shown in Table 1, four groups (groups 2–5) received variation in output energy and repetition rate. Running lasers with a pump energy close to the laser threshold led to instability and a loss of reliability when working at this relatively low output energy. This was resolved by using a higher pump energy (to ensure stability) but with glass slides apposed into the beam path in front of the coupling into the fiber optics to attenuate the beam such that the beam profile at the output end of the fiber tip was unchanged. The actual output energy was measured with an external laser energy meter (Nova; Ophir Optics, Wilmington, MA). In the control group (group 1), neither NaOCl exposure nor laser activation took place. These samples served as reference for a pre-dissolution tissue-filled canal. The fiber tip was immersed to the full length of the canal (20 mm) then immediately withdrawn as far as the upper edge of the root canal model. This process was repeated seven times. Laser activation occurred only during withdrawal of the fiber tip away from the apex, which took 15 seconds (withdrawal velocity: 1.33 mm/second). Immersion in the canal without laser activation took 2 seconds. At an output energy of 10 mJ, fluid was noticeably extruded from the canal, so a sealing ring of silicone with a hole through which to insert the fiber was attached to the upper edge of the root canal model with adhesive tape. All experiments were performed by the same investigator to ensure comparability.

The root canal model was placed horizontally with the NaOCl compartment uppermost, and was unscrewed immediately after the experiment. Residual NaOCl was absorbed carefully with paper towels, with care not to disturb the tissue. Results were documented by acquiring pictures of each semi-canal with its residual tissue (Sony HDV Handycam; Carl Zeiss AG, Feldbach, Switzerland).

Damage to the plain-ended fiber tip was assessed after each experiment using a light microscope (Axiophot, Carl Zeiss AG). Where necessary, the damage was repaired with a grinding device consisting of an acrylic glass cylinder with a central drill hole (diameter: 0.3 mm) into which the fiber could be positioned without clearance. This ensured that only the damaged parts were exposed and that the plain end was grained perpendicularly to the tip's axis. The grinding procedure was carried out by hand on wet abrasive paper (grain size: 2,500 and 4,000) and opaline. After grinding, the tip was re-checked under the light microscope.

Quantification of Soft Tissue Dissolution

Pictures were analyzed using ImageJ, a Java-based public domain software tool developed by the National Institutes of Health, USA (available at <http://rsb.info.nih.gov/ij/>). In areas where dissolution had occurred, the acrylic glass surface was visible. To adjust the light absorption of this material, the average maximal *L*-value (CIE Lab Color Space) of the adjacent acrylic glass surface was determined by three measurements in different places

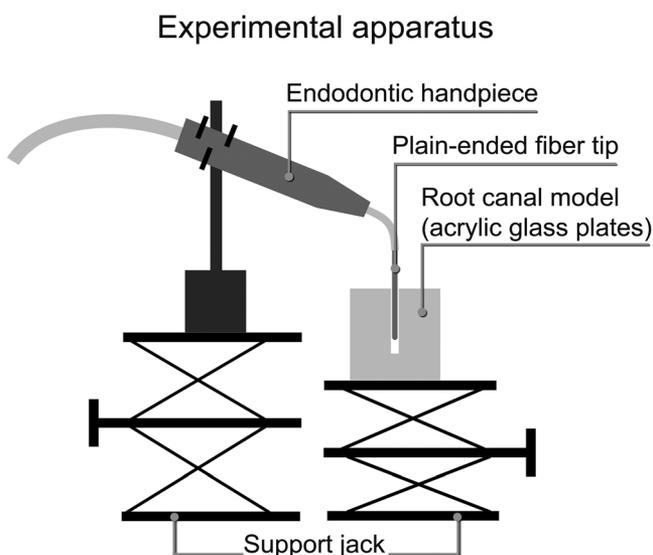


Fig. 1. Experimental apparatus for Er:YAG laser activation of sodium hypochlorite for soft tissue dissolution in an artificial root canal.

TABLE 1. Laser Settings in Each of the Five Experimental Groups

Group	Input energy (mJ; number of glass slide attenuators (gs))	Output energy (mJ)	Repetition rate (Hz)	Laser pulses	Output power (mW)
1 ($n = 2$)	0	0	0	0	0
2 ($n = 5$)	80 (+3 gs)	5	10	1,050	50
3 ($n = 5$)	120 (+2 gs)	10	10	1,050	100
4 ($n = 5$)	120 (+3 gs)	5	20	2,100	100
5 ($n = 5$)	100 (+1 gs)	10	20	2,100	200

for each photo using the histogram function of ImageJ. Subsequently, all areas of the picture except the semi-canal were excluded using Adobe Photoshop CS3 Version 10.0 (Adobe Systems Inc., San Jose, CA) to isolate the region of interest. The L -value was subsequently reduced to the pre-determined individual maximal L -value of the adjacent acrylic surface. Thus, using the "Adjust-Color Threshold" function of ImageJ, pixels of those areas where the acrylic glass surface had been visible were represented as white (L -value of 255). Where deeply blue-colored residual tissue remained, the L -value was less than 255 and the pixels were not filtered out. The relative extent of tissue dissolution was determined as the quotient of the number of pixels where $L = 255$ (dissolved areas) and the total number of pixels of the root canal. Figure 2 shows examples for pictures of root canals filled with dyed soft tissue before and after tissue dissolution, and after adjustment of the light absorption.

Data Analysis

The data were statistically analyzed by two-way ANOVA ($P < 0.05$, univariate, bifactorial; IBM SPSS Statistics 19, SPSS Inc., Chicago, IL).

RESULTS

There were significant differences ($P < 0.05$) in tissue dissolution between all groups, except between groups 3 and 4 and groups 2 and 4. Both output energy and repetition rate had a significant influence on the ability of NaOCl to dissolve soft tissue. The influence of the energy on the relative extent of tissue dissolution was stronger ($\eta^2 = 0.75$) than the influence of the repetition rate ($\eta^2 = 0.56$). Furthermore, there was an almost linear correlation between the energy and the relative extent of tissue dissolution. When the parameters were set to 10 mJ/20 Hz, the resultant tissue dissolution was almost complete (Figs. 3 and 4).

DISCUSSION

A root canal model was established such that (1) it could be filled with soft tissue substitute, (2) a laser fiber tip could be used within the filled canal without scratching soft tissue off the canal walls and (3) the experimental procedure could be observed in its entirety. Previously used root canal models [6,19,27] did not meet these criteria. As in any *in vitro* model, there are limitations compared with the clinical situation: for instance, the smooth root

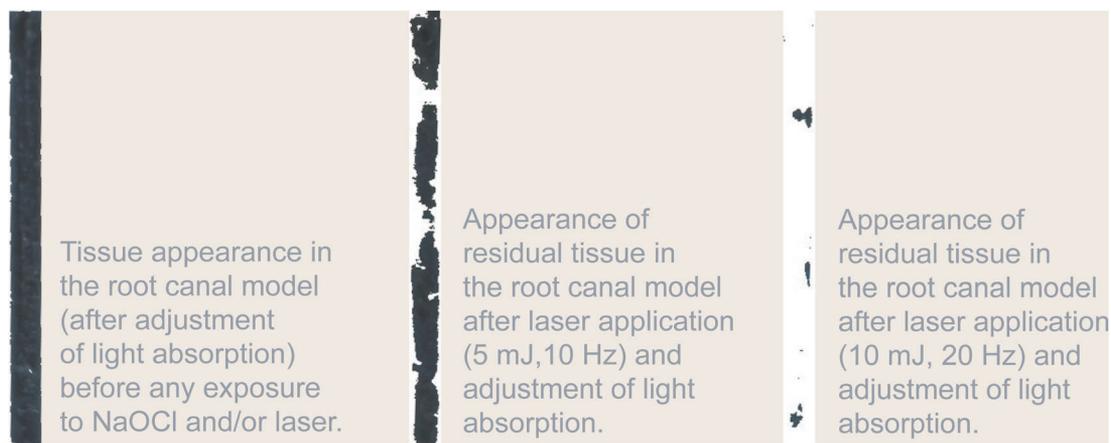


Fig. 2. Dyed soft tissue after adjustment of light absorption. Images show residual tissue and areas of dissolution in the root canal model.

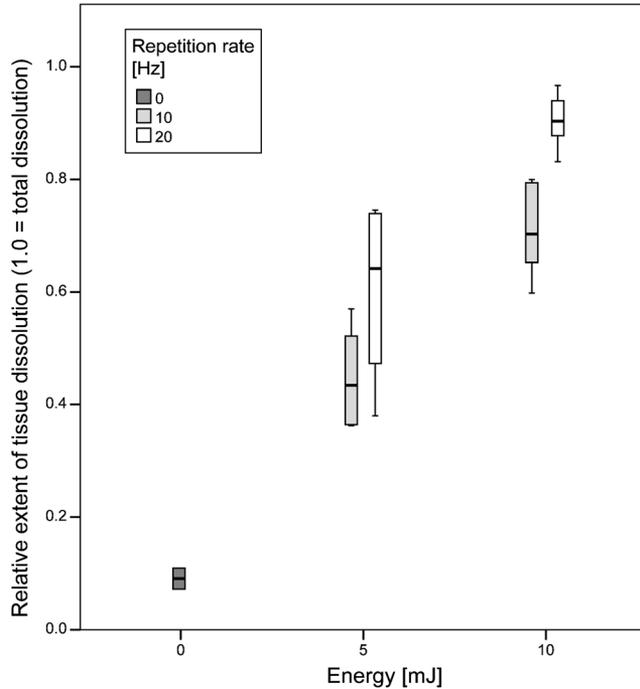


Fig. 3. Influence of laser energy on the relative extent of tissue dissolution by sodium hypochlorite.

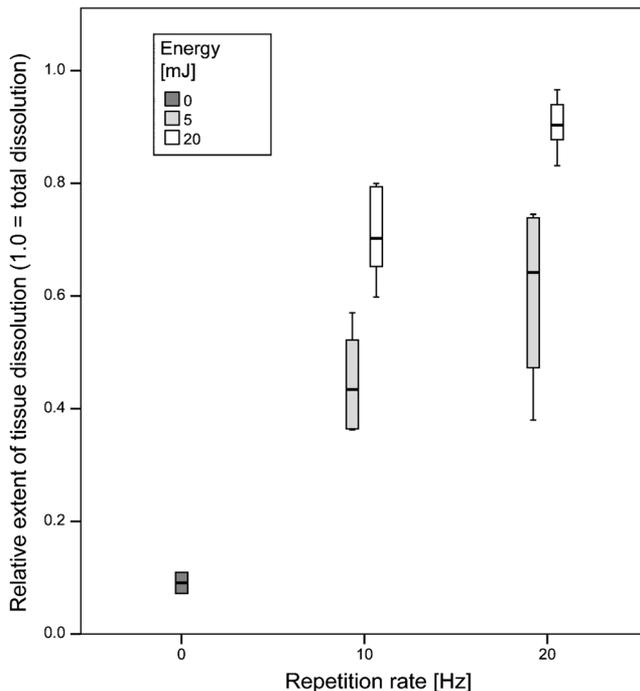


Fig. 4. Influence of the repetition rate on the relative extent of tissue dissolution by sodium hypochlorite.

canal walls in the model compared with the non-uniform surface of the root canal *in vivo*.

The effectiveness of the laser application was investigated using a relatively low output power. Primarily, the procedure should be such that its use in clinical practice is feasible. Many studies using higher power settings reported flushing of the rinsing solution from the canal because of the high-speed fluid motion [21,22], a phenomenon that was also observed in this study. The uncontrollable nature of this fluid ejection at high output power represents a significant safety problem for both the patient and dental staff given the corrosiveness of this irrigant. Furthermore, the risk of an apical extrusion of irrigant induced by laser activation [27] should be reduced by decreasing the laser parameters. Additionally, a safety distance to the ablative threshold seems to be advisable, as collateral damage could be produced by direct contact of the fiber tip with the root canal wall [23], which cannot be excluded in practice. Vapor bubbles arose at very low energy and repetition rate parameters and continued to occur when these parameters were increased. Preliminary studies detected vapor bubble formation in cases where this laser system was used in distilled water with a plain-ended fiber tip at an output energy of 0.36 mJ or higher. Furthermore, the length of the vapor bubbles increases linearly with higher output energies [22]. If there is a great discrepancy in the dimensions of the canal and vapor bubbles, the bubbles could fill the canal. Blanken et al. [22] showed that almost the whole canal was filled with vapor at their settings. The rinsing solution was displaced upwards and downwards towards the apical region. To make the fluid motions within the canal more predictable, these bubbles must be diminished by using a low output energy.

In this *in vitro* study, the fiber was immersed to the full canal length (20 mm), whereas in real teeth with an apical foramen, a safety distance from the apex is recommended [21,22,28], which will vary depending on the length of the vapor bubbles and thus on the laser settings. Preliminary studies using this laser system with the plain-ended fiber tip in distilled water showed that vapor bubbles had a length of 1.4 mm at an output energy of 10 mJ. However, to reliably predict a suitable safety distance, further studies are required to characterize bubble creation in sodium hypochlorite in the appropriate geometrical environment at these same laser settings.

Because the ejection of NaOCl is a safety problem, a further safety device similar to the sealing ring should be developed to prevent NaOCl from extruding coronally from the canal, unless the suction protocol is rigorous and fail-safe. In practice, one cannot prevent irrigant from escaping from the canal, for example into the cavum dentis. Therefore, the use of copious irrigation to refresh irrigant levels between laser activations, according to the common protocol, is to be recommended. This is also important to refresh the available chlorine. Macedo et al. [29] showed in an *in vitro* study that laser activation is a strong modulator of the reaction rate of NaOCl, increasing the consumption of available chlorine significantly. As the quantity of

available chlorine is essential for tissue dissolution, regular exchange of NaOCl to maintain optimal available chlorine levels could further increase tissue dissolution. However, a conventional rinsing protocol with continuous exchange of rinsing solution was not included in this study.

The beam profile of a plain-ended fiber tip can be described as forward-emitting and slightly divergent. The sidewalls in a root canal are not directly affected. Instead, the endangered apical region, where irrigant could escape, is most affected. Nevertheless, we chose this geometry because the use of the fiber in the canal caused damage to the tip and, upon checking for damage after each experiment, restoration of the tip to this geometry was both more time- and cost-efficient. Repair is critical because the effectiveness and safety of the treatment is no longer guaranteed if damaged fiber tips are used since this damage changes their beam profile [30]. Efforts have been made to create side-firing fibers that seem more suitable for application to the endodontic field [31]. The output power level of such side-firing tips would necessarily be lower when using the same laser settings, so re-optimization of the settings would be required to achieve comparable results.

The quantification of soft tissue dissolution has been carried out in several ways, for example by the evaluation of weight loss [2,3] or spectrophotometric analysis [1]. In this study, a new optical quantification technique based on ImageJ analysis software has been developed. The efficacy of this software for image processing and analysis has been demonstrated in many publications describing the analysis of ultrasonographic images [32], the quantification of fluorescence signals [33], adipose tissue [34], and leukocyte adhesion [35].

Both output energy and repetition rate had significant influence on the extent of tissue dissolution which is probably due to a positive modulation of the reaction rate of NaOCl [29] and fluid motion effects. Vapor bubbles are created by local heating and vaporization effects. Secondary cavitation effects [21,22] are also induced and imploding bubbles result in strong liquid turbulence, which is likely to augment soft tissue dissolution.

CONCLUSION

Within the limitations of this *in vitro* study, we conclude that laser activation of NaOCl at an output power of 200 mW generates highly effective soft tissue dissolution.

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